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Zerumbone: A Natural Compound with Anti-Cholinesterase Activity

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Abstract: Herbal dugs could be a new source for inhibitors of acetyl cholinesterase (anti-AChE), the key enzyme in the breakdown of acetylcholine and a new talented approach for the cure of elderly neurologically associated disorders such as Alzheimer's disease (AD). Zerumbone (ZER) is sesquiterpene from the edible plant, *Zingiber zerumbet* which is known to possess tremendous biological activities. In this study, the inhibitory effect of ZER towards acetyl cholinesterase was evaluated using thin layer chromatography (TLC) bioautography and compared concurrently to tacrine, as positive control. The results obtained in this research showed that ZER has an enzymolytic effect towards AChE. It could be suggested that ZER might be a potential candidate for the development of anti-AD treatment.

Key words: *Zingiber zerumbet*, zerumbone, anti-cholinesterase drugs, TLC bioautography

INTRODUCTION

Alzheimer's Disease (AD) is the most common cause of senile dementia in elderly population and is estimated to account for 50-60% of dementia cases in persons over 65 years of age[1]. It is also estimated that up to 4 million of people are affected in the USA[2]. Recently, it has been noticed that research about AD has allowed and strengthened a substantial progress in the physiological and clinical understanding of this pathologic condition[3]. This progress has also opened new windows for the research on the cure of AD[4]. Thus, new treatment approaches have been investigated such as the anti-cholinesterase compounds[5]. One of the richest resources for new anticholinesterase drugs are natural products[6]. *Zingiber zerumbet* (L.) Sm., known as lempoyang among the Malays, is a member of the family *Zingiberaceae*. ZER is a crystalline monocyclic sesquiterpene derived this plant. This bioactive component has its unique structure, with cross-conjugated ketone in an 11-membered ring, as well as interesting biological activities. It has been reported that the compound ZER constitute about 37% of *Z. zerumbet*[7]. To screen the anticholinesterase activity of ZER an effective and fast assay system was adopted by utilizing silica gel Thin-Layer Chromatography (TLC), a qualitative method for AchE activity measurement on a TLC plate[8].

MATERIALS AND METHODS

ZER and sample preparation: ZER was isolated using hydrodistillation method. Briefly, the fresh plant of *Z. zerumbet* was sliced and placed in flask and heated using Mentel heater. This flask was connected with a special glass ware (Dienstag), to collect the volatile oil of the boiled plant material. The volatile oil was crystallized using circulating cool water. Crystals were collected. To obtain a pure material of ZER, recrystallization was performed using hexane. Purity and structure of ZER was verified using liquid chromatography mass spectrophotometry and NMR, respectively. ZER was prepared as a solution of 1 mg mL^{-1} in methanol.

TLC bioautography of acetylcholinesterase enzyme:
Buffer: Phosphate buffer (50 mM, pH 8). 1.56 mg of NaH_2PO_4 in 200 mL.

Acetylcholinesterase enzyme: Acetylcholinesterase enzyme (Lyophilized powder) was dissolved in 20 mL of the phosphate buffer (6 U mL^{-1} as working solution). The stock solution was kept at 4°C (349 U mL^{-1}). Tacrine was used as a positive control (10 mM).
Substrate: Aceytolthiocholine iodide (ATCI) was prepared by dissolving 2.892 mg of ATCI powder in 10 mL buffer to get a working solution of 1 mM.

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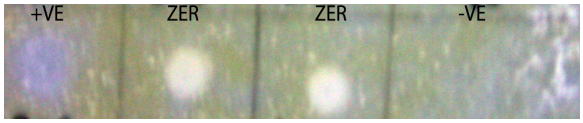

Fig. 1: Bioautograph showing the inhibition of acetyl cholinesterase activity by ZER (1 mg mL^{-1}) and positive control tacrine (10 mM). This bioautographic assay was conducted using thin layer chromatography plates

DTNB (dithiobisnitrobenzoate): DTNB was used as color developer and prepared by dissolving 3.964mg of DTNB powder and dissolved in buffer.

Assay procedure: Five µL of ZER solution was spotted on the TLC plate and developed in chloroform: methanol (8:2), then this solvent mixture was dried and sprayed with DTNB/ATCI. The plate then was left to dry for 5 min. The enzyme was sprayed and observed after 5 min. A yellow background appeared, with spots for the active compound. These were observed and recorded within 15 min because they disappeared in 20-30 min[6]:

$$\text{Acetylthiocholine} + H_2O \xrightarrow{\text{Ache}} \text{acetate} + \text{thiocholine}$$

Thiocholine + DTNB ↔ 5-thio-2-nitrobenzoate (yellow color) + 2-nitronitrobenzoate-5- mercaptothiocholine.

RESULTS

ZER (1 mg mL^{-1}) and positive control tacrine (10 mM) were developed on a TLC plate with the solvent system chloroform:methanol 8:2. These bio-autography results were obtained using TLC. Whereby, AchE inhibiting spots seen after spraying the substrate. Bioautograph obtained in this research shown that ZER has a potential anti-cholinesterase activity which could be visualized clearly in Fig. 1. Comparatively, tacrine (10 mM), as apostive control has given a similar result and there was no spot notice for negative control.

DISCUSSION

Nowadays, natural product research is leading to obtain promising drugs in curing human ailments. And now it becomes one of the most challenges to modern pharmaceutical industry. Plant derived compounds have been plays an important source to several synthetic drugs[9]. Since AD, one of the most common cause of death worldwide, has become a threaten to public health, new treatment strategies based on medicinal plants have been focused[10,11]. The ability of ZER to inhibit AChE by has been evaluated through TLC Bioautography and compared concurrently to the tacrine, as positive control. The results obtained in this study propose that ZER might be a potential candidate for the development of anti-AD drug isolated from edible plant[12].

CONCLUSION

The results obtained in this investigation illustrated that ZER has an enzymolytic effect towards AChE. It could be suggested that ZER might be a potential candidate for the development of anti-AD treatment.

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